techniques to measure blood pressure, lack of measurements of diastolic or mean pressures, failure to control intake of food and NaCl, use of anesthetics, or the use of an inadequate sample size. The tail-cuff technique, the method most commonly used to measure arterial blood pressure in the unanesthetized rat, requires restraint of the animal and either the deliberate or inadvertent induction of an increase in body temperature (*15*). Thus, hemodynamic effects of heat stress or immobilization stress may also have contributed to the variability in blood pressure results. Furthermore, this technique is generally used to measure only systolic pressure because tail-cuff measurements of diastolic or mean pressure are probably not as accurate or as reliable as those of systolic pressure (*15*).

In those studies in which blood pressure in young S rats has been found to be greater than in R rats despite a "low" NaCl diet, the amounts of sodium chloride provided were actually more than adequate for normal growth (*6, 9, 10–12*). Whether or not the stress of tail-cuff measurements or of anesthesia contributed to the occurrence of greater blood pressure in the S rats was also not addressed in those studies.

Our finding that blood pressure in the recently weaned S rat is greater than that in the R rat, despite a diet deficient in NaCl, has implications for studies of the pathogenetic determinants of salt-sensitivity and salt-resistance in the Dahl rat. Specifically, biologic differences demonstrated between the S and the R rat after weaning, including the phenomenon of salt-sensitivity, could be a consequence of, or be dependent on, an already extant difference in blood pressure between the two strains. The observation that, in the weanling S rat, heart weight is greater than in the weanling R rat suggests that the greater blood pressure in the young S rat has biologic consequences, for example, myocardial hypertrophy.

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A New HTLV-III/LAV Encoded Antigen Detected by Antibodies from AIDS Patients

Abstract. *A newly identified protein from HTLV-III/LAV, the virus implicated as the etiologic agent of the acquired immune deficiency syndrome, was studied. This protein, which has a molecular weight of 27,000 (p27), was shown by amino acid sequencing to have a coding origin 3' to the* env *gene on the HTLV-III genome. The presence of antibodies to p27 in virus-exposed individuals indicated that this gene is functional in the natural host.*

JONATHAN S. ALLAN
Department of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts 02115
JOHN E. COLIGAN
Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20205
TUN-HOU LEE
MARY FRANCES MCLANE
PHYLLIS J. KANKI
Department of Cancer Biology, Harvard School of Public Health
JEROME E. GROOPMAN
Department of Medicine, New England Deaconess Hospital, Boston, Massachusetts 02115
MYRON ESSEX
Department of Cancer Biology, Harvard School of Public Health

Acquired immune deficiency syndrome (AIDS) is a disease that causes depletion of a subset of T lymphocytes and results in opportunistic infections and malignancies. A retrovirus, designated HTLV-III/LAV, has been isolated and implicated as the etiologic agent for AIDS (*1–6*). Proteins specific to HTLV-III/LAV have been described and include the *gag*-encoded products p55, p38, p24, and p17 and the *env*-encoded proteins gp160, gp120, and gp41 (*3, 4, 7–11*). Analysis of DNA provirus clones led to the identification of at least two open reading frames, in addition to *gag, pol,* and *env*, that could encode other protein products (*12–15*). A protein of approximately 27 kilodaltons (p27), which appeared to be unrelated to the known *gag*- and *env*-encoded products, was detected in an HTLV-III–infected cell line, H9/HTLV-III (*9*). This protein was analyzed to determine if it originated from one of the open reading frames.

The relative frequency of antibodies to p27 in the serum of patients with AIDS-related complex (ARC) or AIDS and healthy asymptomatic homosexuals was examined. Disease categories were determined according to the criteria for AIDS used by the Centers for Disease Control (*16*). The AIDS patients included those who had clinical manifestations of opportunistic infections or certain malignancies, and ARC patients included those with defined constitutional and laboratory abnormalities. Serum samples, obtained from 138 individuals, were selected at random from a bank of samples drawn from area hospitals and community clinics. These samples had previously been determined by radioimmunoprecipitation (RIP) and cell membrane immunofluorescence to contain antibodies reactive with HTLV-III/LAV proteins. Of the 138 samples tested by RIP analysis, 51 (37 percent) were positive for antibody to p27. The prevalence rates by disease category are given in Table 1. Approximately one-half of the samples from asymptomatic subjects who were positive for HTLV-III/LAV antibody also had antibodies to p27, but this frequency significantly declined with sever-

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ity of disease. Whether this apparent loss in reactivity to p27 is simply a function of antibody titer or alternatively reflects a more profound differential loss of antibodies to p27 is not known.

Figure 1 shows the results of representative HTLV-III/LAV positive serum samples that were analyzed by RIP and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) with [^{35}S]cysteine-labeled H9/HTLV-III cells. Of the serum samples represented, four recognized p27 (lanes 2, 3, 4, and 11), the first three from ARC patients and the fourth from an AIDS patient. Representative negative control sera from people not within AIDS risk groups did not recognize this protein (lane 1), nor did serum samples from other ARC patients (lanes 5, 8, 9, 10, and 13), from AIDS patients (lanes 6 and 12), or from a representative healthy homosexual male (lane 7).

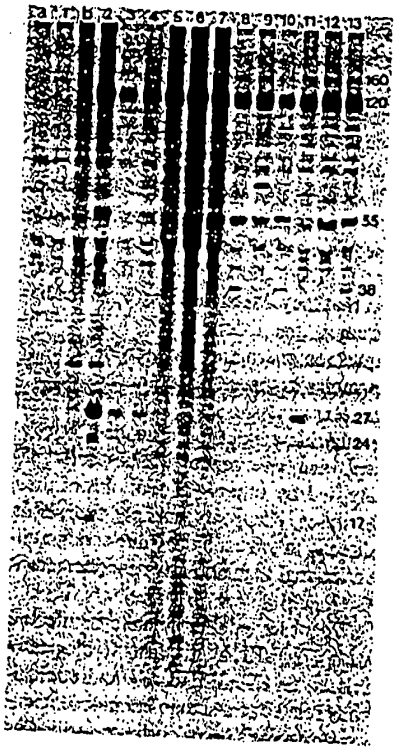
The spectrum of reactivity of serum samples to HTLV-III encoded proteins suggested the possibility that p27 might represent another class of HTLV-III/LAV products unrelated to the proteins encoded by the *gag* or *env* genes. As shown in Fig. 1, one group of serum samples tested recognized *env* and p27 without reacting to the *gag* gene products (lane 3), whereas others reacted with *env* and *gag* antigens as well as p27 (lane 2). An additional group reacted with *env* and *gag* antigens but not p27 (lanes 8, 9, 10, and 12), and still others reacted only with *env* antigens. The possibility that p27 was not a *gag* or *env* protein was consistent with the observation that p27 was not precipitated by antibodies to *gag* proteins, and it was not glycosylated as are those encoded by the *env* gene (*17*).

Table 1. Prevalence of antibody to p27 among HTLV-III positive individuals. The status of individuals was determined with the CDC criteria for AIDS. All sera were selected from a bank of serum samples previously evaluated by indirect membrane immunofluorescence and RIP/SDS-PAGE. All 138 samples contain antibodies reactive to *env* products (*9, 10, 16*).

Status	Ratio of number positive* to number tested	Percent positive
Healthy homosexuals	13/27	48.1
ARC	18/42	42.9
AIDS	20/69	29.0
Total	51/138	37.0

*Samples were tested by subjecting [^{35}S]cysteine-labeled H9/HTLV-III cells to RIP/SDS-PAGE as described (*7, 9, 10*).

To determine the coding region of p27, we analyzed the amino-terminal amino acid sequence by radiosequence analysis (*18–20*). Radiolabeled p27 was prepared by immunoprecipitation of [^{3}H]leucine- or [^{3}H]arginine-labeled H9/HTLV-III cell lysates with standard positive reference serum. Protein precipitates were then subjected to SDS-PAGE. Automated sequence analysis did not provide information on the presence of either of these amino acids in the first 35 degradation cycles at the amino terminus of the protein. This result indicated that either there were no leucine or arginine residues in the first 35 amino acids of the amino terminus, or that the amino terminus of p27 was inaccessible to Edman degradation. We therefore prepared cy-



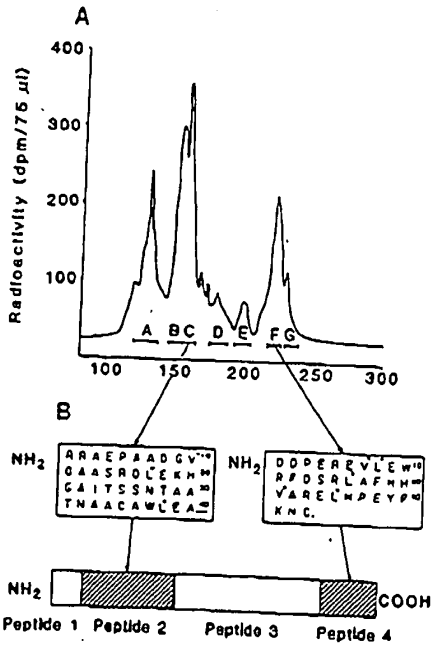


Fig. 1 (left). Antibody reactivities to HTLV-III–specific p27. Cell lysates were prepared from [^{35}S]cysteine–labeled H9/HTLV-III cells or H9 uninfected cells. Details of the RIP and SDS-PAGE techniques were described earlier (*7, 9, 10*). Lysates were immunoprecipitated with the following sera: Normal human serum was tested on uninfected H9 cells (lane a) and on H9/HTLV-III cells (lane 1); positive reference serum from an ARC patient was tested on uninfected (lane b) and infected cells (lane 2). Seven ARC patients (lanes 3, 4, 5, 8, 9, 10, and 13), three AIDS patients (lanes 6, 11, and 12), and one healthy homosexual male (lane 7) were tested on infected H9/HTLV-III cells. Immunoprecipitates were subjected to electrophoresis on 20-cm 10 percent SDS-polyacrylamide gels. Fig. 2 (right). Amino acid sequence analysis of cyanogen bromide peptides from p27 and comparison to the deduced protein sequence of 3′ *orf*. (A) Separation of [^{3}H]leucine-labeled CNBr peptides of p27 by Sephacryl S-200 chromatography. A similar profile was observed for [^{3}H]valine-labeled peptides. (B) Amino-terminal amino acid sequence analysis of CNBr peptides. Pools C and F were subjected to Edman degradation, as shown in Fig. 3, and the sequence was compared to the predicted amino-terminal sequence of CNBr peptides from 3′ *orf*. The labeled amino acids and their positions are designated by asterisks. A schematic representation of the 3′ *orf* gene product and placement of the predicted peptides is shown. Predicted CNBr cleavage sites and resulting peptides are based on methionine residues at positions 1, 20, 60, and 170 of the 204-amino acid gene product. H9/HTLV-III cells were metabolically labeled with 10 mCi of [^{3}H]leucine or [^{3}H]valine for 4 hours, and cell lysates were prepared. Radiolabeled lysates were precipitated with standard HTLV-III reference serum (same as Fig. 1, lane 2) and subjected to electrophoresis on 10 percent SDS-polyacrylamide gels. The p27 was excised from the gel, electroeluted, and lyophilized before CNBr cleavage. Cyanogen bromide hydrolysis of p27 was accomplished by incubating the protein for 9 hours with 2 percent CNBr in 70 percent formic acid. The reaction was terminated with the addition of nine volumes of water, and the mixture was lyophilized. Sephacryl S-200 separation of CNBr peptides and automated sequence analysis were performed as described (*18, 20*). Fractions were collected, assayed for radioactivity, pooled, and subjected to Edman degradation. The sequences for pools C and F were matched to the deduced amino acid sequence of the predicted 3′ *orf* CNBr peptides.

anogen bromide fragments from [^{3}H]leucine- or [^{3}H]valine-labeled p27 for amino acid sequence analysis. The cyanogen bromide fragments were first separated by Sephacryl S-200 chromatography and the fractions were pooled (Fig. 2). Of the pools analyzed by Edman degradation, pools C and F contained materials that gave reactive peaks with greater than 91 percent repetitive yield. Leucine peaks were observed at positions 17 and 38, and a valine peak was observed at position 10 of pool C. Pool F contained leucine peaks at positions 8, 16, and 25 and valine peaks at positions 7 and 21 (Fig. 3). The predicted amino acid sequence for the 3′ open reading frame of HTLV-III specifies four peptides that are generated from cleavage by cyanogen bromide at methionine residues (*12–15*) (Fig. 2). When we compared the predicted amino terminal amino acid sequences of these peptides with leucine and valine profiles of the peptides analyzed, we found that the sequence derived from pool C matched that of a predicted peptide designated peptide 2. In addition, the sequence derived from pool F matched the predicted cyanogen bromide peptide designated peptide 4. A summary of the predicted amino acid sequence for the peptides for p27 is illustrated schematically in Fig. 2B. The probability of finding such a match by chance alone is less than 2.9×10^{-6} for pool C and 2.09×10^{-8} for pool F. The results enable us to assign the coding origin of p27 to the 3′ terminal region of HTLV-III.

We have thus described p27, a previously unrecognized HTLV-III specific antigen that is capable of generating an immune response in the natural state as evidenced by the recognition of this protein by the antibodies from the sera of patients with AIDS and ARC and that of healthy exposed individuals. By assigning its coding region to the 3′ region of the HTLV-III genome, we provide direct evidence for the presence of a fourth functional gene located 3′ of the *env* gene.

Because of inaccessibility of the amino terminal sequence of p27 to Edman degradation, the exact genomic location of the methionine initiator codon could not be unequivocally assigned. We observed that p27 undergoes posttranslational modification by myristylation as evidenced by its incorporation of [^{3}H]myristate (*17*). Earlier studies on retroviral proteins with such posttranslational modification revealed that the amino terminus was "blocked" for Edman degradation and that the initiator methionine residue was followed by a glycine (*21–23*). If the myristylated p27 also has an amino terminal protein sequence that conforms with the Met-Gly consensus sequence, the first AUG codon in the 3′-*orf* products which begins five nucleotides 3′ from the end of the *env* gene and is followed by a glycine codon is a likely initiation point for p27. This conclusion is in agreement with previous analyses of major transcripts detected in the HTLV-III–infected cells (*15, 24*).

The estimated molecular weight of p27, as determined by SDS gels, however, could be interpreted as a discrepancy because it is larger than the size predicted for a 3′ open reading frame protein, which is composed of about 200 amino acids (*12–15*). It seems likely that the myristate attached to p27 may have caused an abnormal migration pattern in SDS gels. A similarly misleading migration in SDS gels due to myristylation has been observed with the *gag*-encoded p19 of HTLV-I, which migrates as a 19-kd protein yet contains only 126 amino acids (*25*).

Detection of antibodies to p27 among those who are seropositive for HTLV-III indicates that there are at least four different classes of HTLV-III–encoded antigens that may elicit immune responses during a natural course of HTLV-I infection. We and others have shown that antibodies to *gag*- and *env*-encoded products can be detected in HTLV-III infected individuals (*3, 4, 7–11*). Recently, antibodies to the carboxyl terminus of the *pol* gene product were also found in AIDS patients (*26*). Judging from the antibody recognition pattern, we previously concluded that when *gag* and *env* products were compared, *env* products were the most immunogenic species (*9, 10*). Because p27 was recognized by only 37 percent of those who were seropositive for other antigens, p27 cannot be considered to be as immunogenic as the antigens encoded by the *env* gene. However, the frequency of antibody to p27 in AIDS patients is lower than that for ARC patients and healthy homosexual males combined (χ^2 d.f. (1) = 3.73, $P = 0.05$). This aspect of antibody response is the same as that directed to the *gag* encode p24, in that AIDS patients are more likely to lack detectable circulating antibodies to antigens other than those encoded by the *env* gene (*9*). The biological significance of the selective absence of circulating antibodies to p27 or p27 (or both) in AIDS patients is not known. It does point out, however, that these antigens will not be as valuable as *env*-encoded products for diagnostic purposes.

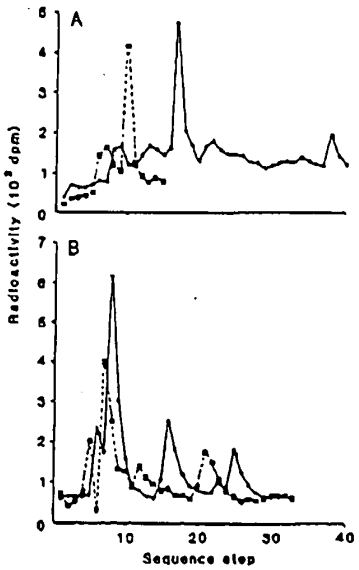


Fig. 3. Analysis of the amino terminus of p27 radiolabeled CNBr peptides. [^{3}H]leucine- (●) and [^{3}H]valine (■)-labeled p27 CNBr peptides were separated by Sephacryl S-200 chromatography (Fig. 2) and subjected to semiautomated Edman degradation as described (*20*). Radioactivity is shown for each degradation cycle. (A) For pool C, leucine peaks were observed at positions 17 and 38 and a valine peak at position 10. (B) Pool F contained leucine peaks at 8, 16, and 25 and valine peaks at 7 and 21. The relations of pool C and F to the predicted amino acid sequence of 3′ *orf* is given in Fig. 2.

Proteins that are encoded by sequences extending into the 3′ long terminal repeat (LTR) of HTLV-I and HTLV-II, such as p42 and p38 (*18, 27*), function as transcriptional regulators. The coding sequence of p27 resembles p42 and p38 of HTLV-I and HTLV-II, respectively, in that they are encoded by open reading frames that extend to 3′ LTR's. The question of whether similarities of these proteins in their structural arrangement at the genomic level imply functional similarities, like those encoded by *gag*, *pol*, and *env* genes, was recently addressed by others (*24, 28*). From their analyses, it appears unlikely that p27 is directly involved in the *trans*-acting transcriptional regulation as described earlier (*28, 29*).

We observed that monkeys infected with STLV-III, a retrovirus serologically related to HTLV-III/LAV and etiologically related to a simian immunodeficiency syndrome (*30*), also have antibodies to p27 of HTLV-III/LAV (*31*). This implies that a product identical or serologically related to p27 is also encoded by STLV-III. The evolutionary conservation of gene products encoded by these recently identified human and simian retroviruses warrants further studies on their roles in viral pathogenesis.

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Electrokinetic Separation of Chiral Compounds

Abstract. *Femtomole amounts of racemic mixtures of derivatized amino acids were resolved and analyzed rapidly in about 10 minutes by means of high-voltage zone electrophoresis with laser-fluorescence detection. The electrophoresis was performed in capillary columns containing a chiral support electrolyte. A number of dansyl amino acids were resolved by the diastereomeric interaction between the DL-amino acid and the copper(II) complex of L-histidine present in the support electrolyte. A combination of electro-osmotic and electrophoretic action caused all species, positively charged, neutral, and negatively charged, to pass through the 0.5-nanoliter detection volume where they were subjected to laser excitation.*

E. Gassmann
J. E. Kuo
R. N. Zare
Department of Chemistry,
Stanford University,
Stanford, California 94305

As first pointed out by Pasteur (*1*), one approach to the resolution of a racemic mixture into its component enantiomers is the formation of diastereomeric pairs by the addition of some chiral agent, since diastereomers may have widely different physical properties. This approach is the basis for chromatographic separations of enantiomers by means of chiral stationary or mobile phases (*2*). We report an improvement in this technique in which rapid resolution of a mixture of optical isomers is achieved with unprecedented sensitivity. The improvement results from the combination of high-voltage electrophoresis in a capillary column containing a chiral support electrolyte with laser fluorescence detection. By this method we have resolved in less than 10 minutes a number of DL-amino acids that have been labeled with the highly fluorescent dansyl group.

The use of optically active copper(II) complexes for the chromatographic resolution of enantiomeric amino acids was first demonstrated by Davankov and his colleagues (*3*). For this purpose they immobilized L-proline on resins onto which Cu(II) metal ions were also loaded. Later it was shown by Hare and Gil-Av (*4*) as well as by Karger and coworkers (*5*) that optically active chelate additives in the mobile phase of high-pressure liquid chromatography (HPLC) resolved racemic mixtures into their constituent enantiomers. The optically active Cu(II) L-histidine complex used in our work was introduced by Lam *et al.* (*6*) for the separation of DL-dansyl amino acids by means of reversed-phase HPLC.

An alternative to liquid chromatography is high-voltage zone electrophoresis in capillary columns. As pointed out by Jorgensen *et al.* (*7*) and Terabe *et al.* (*8*), this new analytical tool combines high theoretical plate numbers—on the order of 1 million—with short analysis times.

We used a fused-silica capillary column (a gift from Hewlett-Packard) that was 75 cm in length and had a 75-μm inner diameter. The applied electric field was 300 V/cm, and the support electrolyte contained 5 m*M* L-histidine, 2.5 m*M* $CuSO_4 \cdot 5H_2O$, and 10 m*M* ammonium acetate adjusted to *p*H 7 to 8 by the addition of NH_4OH. The measured current was 30 to 33 μA.

The formation of a double layer on the inside surface of the capillary caused an electro-osmotic flow. Under our experimental conditions, the electrolyte solution moved toward the cathode. Therefore, cations, neutral species, and anions injected at the anode end of the column could be detected at the cathode end in a single run. The analysis was accomplished with an on-column fluorescence detector that had a helium-cadmium laser set at 325 nm (5 mW) as an excitation source. The filtered output of the laser was focused on an optical fiber that carried the excitation light to the on-column flow cell, which had a volume of ~0.5 nanoliter. The resulting fluorescence was collected at right angles to both the excitation direction and the capillary by a second optical fiber that led to a combination of a fast monochromator and a photomultiplier. The dansylated amino acids were either purchased (Sigma) or prepared by known methods (*9*).

Table 1. Migration times (t_D, t_L), Δt values (Eq. 1), and relative peak areas (A_D, A_L) for some DL-dansyl amino acids, measured under the conditions explained in the text, at *p*H 8.0. Abbreviation: DNS, dansyl.

Amino acid	t_D (minutes)	t_L (minutes)	Δt (× 100)	A_D	A_L
di-DNS-Tyr	6.30	6.36	−0.95	1.5	1.8
DNS-Met	6.75	6.71	0.63	1.6	1.6
DNS-αAB*	6.83	6.75	1.2	1.3	1.0
DNS-Phe	6.80	6.91	−1.6	0.18	0.36
DNS-Ser	7.0	7.0	0.0	0.46	
DNS-Val	7.40	7.32	1.1	2.1	1.8
di-DNS-cystine	7.90	8.00	−1.3	0.37	0.39
DNS-Asp	9.80	9.95	−1.5	0.18	0.24
DNS-Glu	10.30	10.10	1.9	1.71	1.38
*DNS-cysteic acid†	10.40	10.70	−2.9	0.15	0.29

*N-Dansyl-α-aminobutyric acid. †N-Dansyl-3-sulfoalanine.